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A Hierarchy of Model Systems for Biomaterials Interfaces: Analysis by Electron, Ion and Vibrational Spectroscopies

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Introduction

There are many problems and challenges in materials synthesis and characterization which allow surface chemists to contribute to the global understanding of mechanisms of interactions between materials and biology. Research in our laboratories has involved both the development of analytical (spectroscopic, microscopic, wettability) methodologies for surface chemical analysis and the application to real problems in biomaterials surface chemistry. Using well defined model systems is a means to bridge surface analytical techniques to real applications.

An obvious approach in surface analytical chemistry is to ask questions which involve developing an understanding of information for ***qualitative*** and ***quantitative*** analysis. A third important area unique to surface analysis is the description of the ***sampling depth*** of the method. Understanding the limits of ***imaging*** technologies also is important. The goal from this work is to evolve to the application of static SIMS to real polymer surfaces, to determine structure and reactivity and develop surface structure-property relationships.

The major focus of this paper will be the most recent results from the development of static Time of Flight SIMS. Three results are particularly promising in the analysis of molecular biomaterials presented in this paper.

- ToF-SIMS has allowed the sequencing of covalently bound minimal peptide sequences at fluoropolymer surfaces for neural cell adhesion applications.
- Polymer tertiary structures such as double helices (produced by LBK films of iso-PMMA) and α helices or β-sheet conformations can yield different ion formation mechanisms in polymer ion formation may yield a means to identify the tertiary structure of adsorbed biomacromolecules.

- The kinetics of surface degradation from biodegradable polymer biomaterials can be followed by oligomeric ion distributions yielded from ToF-SIMS analysis.

Static SIMS - Background

Since SIMS is a mass spectrometric method, the understanding of mechanisms of ion formation should lead to a better understanding of qualitative information. Unlike the study of ion formation mechanisms for traditional mass spectrometry, the challenge in SIMS is to relate mechanisms of ion formation in the reactive "selvedge" region or the gas phase to the chemistry (structure, reactivity) of the existing surface of the solid or material under study.

The development of quantitative methods for static SIMS would allow the extension of powerful detection limits and analysis of mixtures and multiple components inherent in SIMS. Finally, combining the imaging ability with a description of the surface sensitivity, or sampling/information depth, would lead to a better means to describe sample heterogeneity in three dimensions.

Our approach has involved moving through a hierarchy of model systems based on thin film preparation techniques; most particularly the use of Langmuir Blodgett film preparation methods (1,2). These techniques allow the construction of **organized assemblies** in monolayer and multilayer structures, where composition, interfacial or surface chemistry and structure can be controlled. This model system approach has been recently popularized by the widespread use of "Self Assembled Monolayers (SAMs)" to model polymeric/molecular surfaces for technical and biological applications by a number of prominent surface chemistry research groups (2,3). SAMs are molecular and polymer systems which chemisorb and orient/pack from solution, without the use of a film balance/transfer device such as the LB film method uses. The interplay of analysis of monolayer (and multilayer) organic and polymeric films is particularly interesting. The study of monolayer systems produced by adsorption, assembly or Langmuir Blodgett methods by SIMS holds exceptional promise both in the analysis of structure and chemistry in monolayers, and, as stated above, for the use of films as model systems to be extend SIMS analysis to more complex molecular and polymeric surface chemistry.

The initial focus in our laboratory has been entirely on the use of the monolayer and multilayer films as known models to explore the information processes in SIMS. Work began in the mid-1980's with a quadrupole static SIMS instrument, which continues in operation currently. In the area of ***qualitative ion formation mechanisms***, with the LB film approach allowing control of surface chemistry and structure, we have shown the sensitivity of formation of molecular ions formed by protonation and deprotonation of acids and bases (typically designated by $(M+H)^+$ and $(M-H)^-$, where M is the neutral molecule) and anionized or cationized molecular species such as $(M+Anion)^-$ or $(M+Cation)^+$ and other related ions to **structure** and **surface chemistry** (4-9). In addition, we have defined the details of damage in multilayer assemblies (5, 10), leading to a more detailed ***quantitative*** approach to molecular ion formation. This allowed us to derive for the first time a method for determining **concentration** information for molecules from molecular ion intensities

(8, 11-16), or most recently, a quantitative assessment of molecular environment, e.g. pH at a surface (15). These types of conclusions from molecular studies have allowed direct extension to the understanding of ion formation from polymer surfaces (6,7). A recent development is the use of the quantitative model to follow the kinetics of polymerization of diacetylene LB films (17).

We believe the development of the quantitative methodology for SIMS to be particularly significant. Static SIMS is notorious for failing two key analytical performance characteristics; the lack of precision and inability to evaluate the accuracy of the method. Our approach has allowed an exploration of using SIMS for quantitative molecular surface analysis. This latter result could allow the determination of kinetics of crosslinking or degradation at polymer surfaces. At present, as described below, we are working hard to evaluate the use of our new quantitative SIMS methodology to study the kinetics of biodegradation of polymer drug delivery materials. The quantitative SIMS method allows for the study of small/trace concentrations in the presence of majority species, and can further the understanding of molecular structure breakers in ordered organic thin films.

Qualitative Analysis

We have recently demonstrated the ability to **sequence covalently bound peptides at polymeric biomaterials surfaces.** In collaborations with researchers at Kodak Research Laboratories, Brown University and the University of Lausanne (Switzerland) School of Medicine, along with Professor Bright's group at SUNY Buffalo, we have synthesized a new class of lithographically surface modified materials, where we have localized so-called ***minimal peptide sequences*** (18) in spatially defined regions on a modified fluoropolymer surface. This work grew out of our patented surface modification chemistry for fluoropolymers (19, 20) and the localization of amine functional siloxane monolayers at the fluoropolymer surfaces (20, 21), which were visualized by imaging ToF-SIMS (22). The latter materials were shown to be extremely interesting for cellular attachment, promoted by unique protein reorganization upon adsorption (23-26). In extending this work, we sought to more carefully define surface chemistries which would trigger specific cellular function through cellular recognition processes. Two specific sequences of interest were the laminen derived peptides: YIGSR and IKVAV. We developed new chemistry for the covalent attachment, and verified the primary sequence was retained upon linking through the use of the ToF-SIMS. Figure 1 shows the structure of YIGSR at the surface of the polymer and the resultant high mass positive ToF-SIMS of FEP-RSGIY with ions labeled according to the typical Biemann scheme (27). These materials were shown to be superior in directing neural cell adhesion and growth (28, 29).

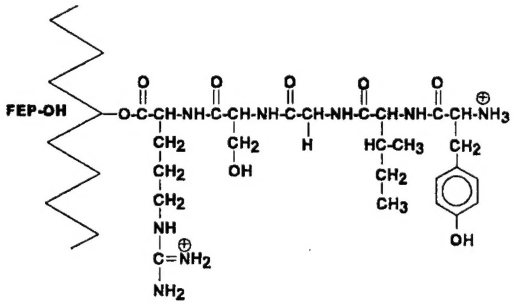
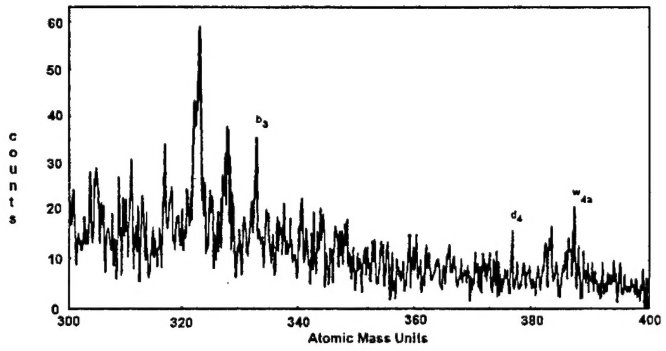


Figure 1a Structure of FEP-RSGIY material at pH = 7.0 (27)



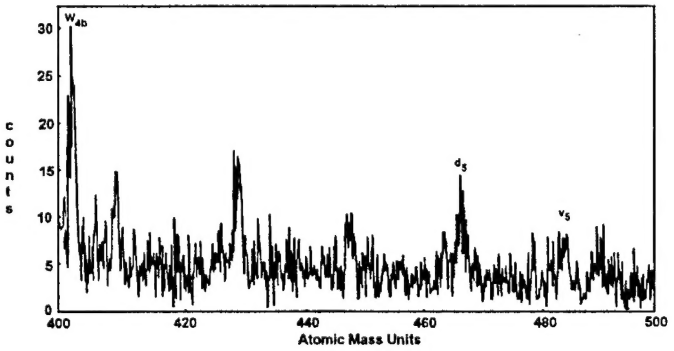


Figure 1b. Positive ToF-SIMS spectra of ions related to peptide structure from FEP-RSGIY (27)

Evolving LB model systems toward more relevant polymer structure questions has been furthered by the recently rediscovered field of LB films of polymers, even those which are not obviously amphiphilic. Some typical examples include common materials like PMMA and PEO, of many polymers which can form monomolecular films at the air water interface in a coiled structure (30, 31). This observation is interesting in light of the capability of certain poly(amino acids), such as poly γ-methyl glutamate, to form monomolecular films at the air-water interface in known (or determinable) tertiary configurations based on α helix or β-sheet secondary structures (31c).

We have been probing whether such resulting monolayer tertiary polymer structure can influence secondary ion formation; if it can, then information about the secondary or tertiary structure of a polymer surface may be extracted from the ToF-SIMS ion formation mechanisms. A startling example of this thesis is illustrated by the results of ToF-SIMS analysis of different secondary structures of PMMA (isotactic, syndiotactic) which yield very different fragmentation patterns at high mass (1000-3000 D) (Figure 2).

The ion patterns from isotactic PMMA could not be explained by consideration of ion formation from statistical chain breaking models. The tertiary structures of the LB preparations were determined using reflection absorption FT-IR (32,33), which has previously shown that isotactic PMMA forms double helical structures in monolayer preparations, similar to that formed in the solid state (34).

Ion formation mechanisms proposing unique rearrangements dependent on the double helical structures have been determined to explain the entire series of ions detected from the iso-PMMA. For example, the scheme shown in figure 3 focusses on a rearrangement site within the closest point of contact of the two chains intertwined in the double helix. A consistent loss of 84D can be used with the previous approach of varying end group rearrangements to explain every fragment in the spectrum of the iso-PMMA.

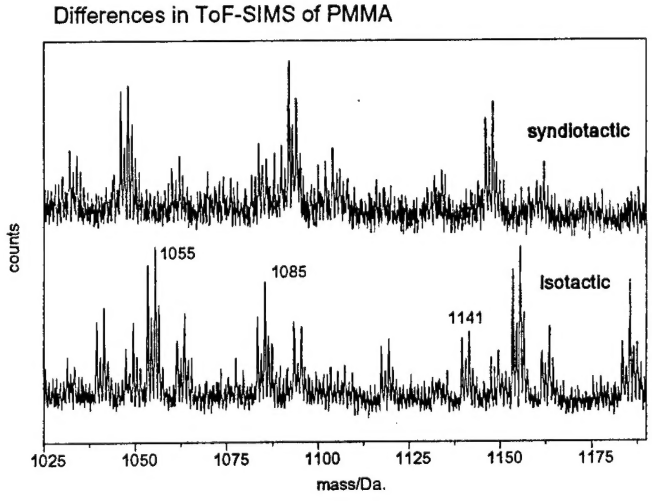
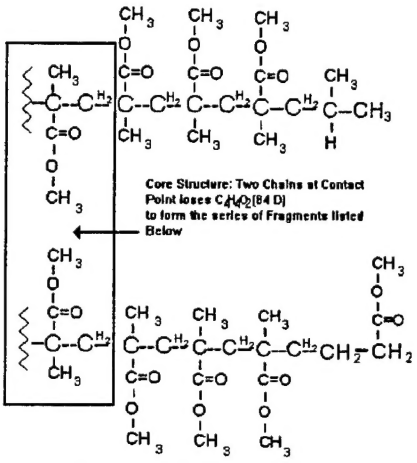
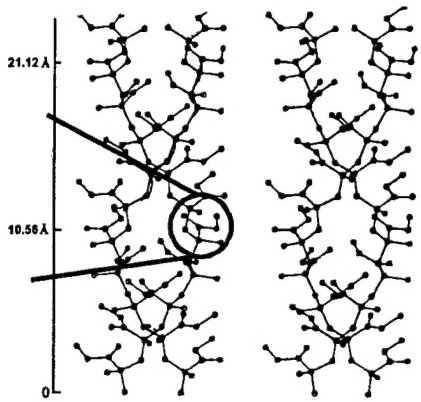


Figure 2 ToF-SIMS Positive Ion Spectrum from(top) syndio PMMA and (bottom) iso PMMA showing repeating ion clusters

Figure 3 (Right) Structure of Iso-PMMA Double Helix in crystal (34) (Left) Scheme of Ion Structures for Iso-PMMA dependant on Double Helical intrachain rearrangement.



FRAGMENTS	
$C_{46}O_{19}H_{82}Ag$	1055.04
$C_{48}O_{20}H_{80}Ag$	1085.02
$C_{52}O_{20}H_{88}Ag$	1141.13



Isotactic-PMMA double-stranded 10/1 helix

Quantitative Analysis

Our newly developed quantitative methodology has been used most recently to study the kinetics of biodegradation of polymer drug delivery materials. In this work, we have studied the simulated biodegradation of poly(glycolic acid) (PGA), (with poly (d-lactic acid) a common component of commercial biodegradable PGA/PLA copolymer sutures and tissue scaffolding material (35). We have followed the extent of surface etching by quantifying the decrease in the molecular weight of degraded (hydrolyzed) chains at the surface of the material. By following the decreasing molecular weight, we have a direct view of the changes in surface chemistry upon etching. Figure 4 shows spectra of samples before and after hydrolysis in a biological buffer system at 37°C for one hour. Note the presence of a new series of ions after the hydrolysis. These ions are directly related to the molecular weight of low mass species created from hydrolysis; and are not present in the unhydrolyzed polymer.

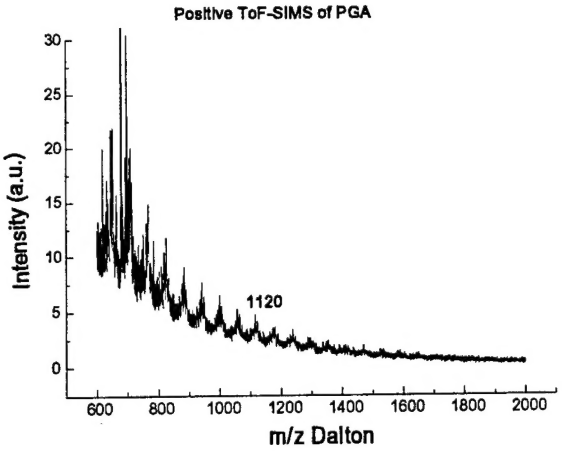
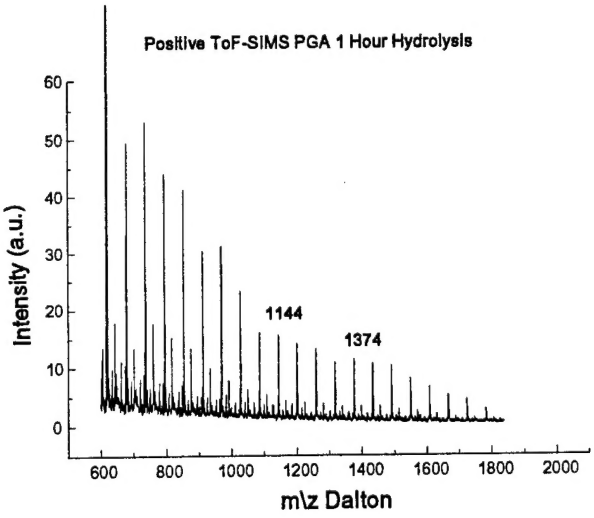


Figure 4 ToF-SIMS of PGA before and after 1 hour hydrolysis in a biological buffer.



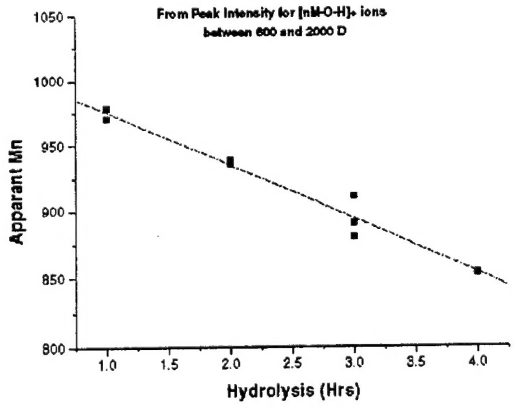


Figure 5 Apparent MWD as a function of degradation time

By calculating an apparent molecular weight for the ions which are apparent after the hydrolysis, we can relate the changes in the molecular weight parameter as a function of time, as shown in Figure 5. This gives us an unparalleled view of the details of the hydrolysis kinetics at the surface of the polymer, something which is only available indirectly in the bulk of the sample by thermal analysis (36).

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